dexamethasone suppression.[4] Therefore, although Chung *et al.* used the dexamethasone-suppression adrenocortical scintigraphy to show the functional activity of the lesions, the diagnosis based only on the scintigraphy could lead to misdiagnosis.[5]

The CT image Chung *et al.* presented showed different contrast enhancement effect in the adrenal lesions, which indicates that the lesions would have different nature. Considering that the existence of adrenal nodular lesions in the patients with hypertension is reported to be not rare,[6,7] it is possible that the only one lesion had aldosterone hypersecretion. Because the small size and the NP-59 accumulation in the case presented by Chung *et al.* indicate the benign nature of the lesions,[6–8] the resection of the only one lesion with aldosterone hypersecretion might have been sufficient.

Adrenal vein sampling is now considered to be the most reliable diagnostic test to detect the site of aldosterone hypersecretion.[9,10] Therefore, we would like to recommend adrenal vein sampling in the cases under the suspicion of primary aldosteronism, such as the case presented by Chung *et al.* The lesions should better be resected after the confirmation of their functional activity.

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# Response to 'Diagnosis of aldosterone producing adenomas'

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**We certainly appreciated the comments by Dr Tanemoto *et al.*[1] regarding our report of Diagnosis of bilateral aldosterone-producing adenomas.[2]**

**The preoperative diagnosis of bilateral aldosterone-producing adenomas remains elusive and can only be definitely made postoperatively by pathological existence of typical adenomas in the excised specimen, and strong expression of the aldosterone synthase by real-time PCR of the excised tumor.[3]**

**Although computed tomography (CT) scanning might not reliably discriminate bilateral adrenal hyperplasia (BAH) from aldosterone-producing adenoma (APA),[4] previous reports showed that the finding of a single-focal macroadenoma on CT scan had a high positive predictive value when the tumor size was $>1.0$ cm;[5,6] and it will be more diagnostic of APA when combined with a positive NP-59 adrenocortical scintigraphy as a valid evaluation tool for primary aldosteronism.[7] Even adrenal vein sampling (AVS), the gold standard of differential diagnosis of the subtypes of primary aldosteronism, has its limitation because of bilateral functional adenomas that may be present at the same time or metachronously. In our institute, there were four cases of simultaneous bilateral aldosterone-producing adenomas among 164 APA patients surgically treated and which were pathologically documented as APAs. These four bilateral aldosterone-producing adenoma patients presented with evidence of bilateral localized uptakes of NP-59 at dexamethasone-suppressed adrenocortical scintigraphy, with CT scan demonstrating tumors larger than 1.0 cm in diameter (1.2–2.0 cm, mean 1.5 cm) and negative postural test before operation. There were another three cases of metachronous bilateral aldosterone-producing adenomas, with the second contralateral tumor clinically detectable 18–48 months after the first adrenalectomy.[8]**

**For this specific patient presented in our report, we also performed real-time PCR of the aldosterone synthase.[2] The mRNA level of aldosterone synthase (CYP11B2) were corrected with the mRNA level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and were quantified using real-time PCR as previously reported,[3] and it is expressed as $2^{-\Delta\Delta CT}$, where $\Delta\Delta C_T = (\Delta C_T$ CYP11B2$-\Delta C_T$ GAPDH). The mRNA level of the bilateral tumorous portions and the nontumorous portions were $\Delta\Delta C_T$, $2.73 \pm 0.13$ versus $12.47 \pm 0.68$ in this patient, indicating much higher mRNA activity in bilateral tumors, and proved that they both are functional APAs. And a radiologist was asked to review the CT scans of that patient again which revealed that his bilateral tumors were larger than 1.0 cm, and both were enhanced with contrast medium.**

**In conclusion, we believed that it is reasonable to perform bilateral subtotal adrenalectomies in treating bilateral APAs, which present with aldosterone–renin ratio $>100$ ng per 100 ml per ng ml$^{-1}$ h$^{-1}$, plasma aldosterone $>20$ ng per 100 ml, and bilateral macronodular tumors ($>1.0$ cm) and positive uptake in the adrenal scan.**

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# Studying channel and receptor physiology in podocytes

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**To the Editor:** We would like to congratulate Shankland *et al.*[1] for their very apt and extensive review regarding the culturing of podocytes in which they have diligently put forth the evolution of this technique. Research in the area of renal glomerular physiology has already seen many technical developments over the past 100 years, and the latter are still evolving. This is mainly due to the complicated and multicellular structure of renal glomeruli and the manner in which their different cells are naturally arranged and interact. In fact, the complexity of glomerular architecture has hindered the progress of detailed and sophisticated research in this area. Cell culturing methods have been a vital ingredient for these advances in renal glomerular research. Podocytes, in culture, present a unique system to study the biology of these cells but, as was acknowledged by Shankland *et al.*,[1] this is not the final frontier. With every advance in research, there is always the need for yet a newer and better model that more closely approximates the actual native subject of interest. Past studies have shown that cultured preparations do not always accurately capture all the features of the *in vivo* conditions. For example, cultured cells have been widely reported to express smooth muscle α-actin, whereas this protein is not found *in vivo* as shown by Egler *et al.*[2] For this reason, it is highly speculative to extrapolate the results obtained in cultured podocytes (which are highly constrained within their cell cycle) to their native *in vivo* counterparts (which are highly differentiating cells).[3] It is far more preferable to work with intact native structures rather than with cultured cells, in order to minimize this confounding factor. With this in mind, we have been optimizing a system to look at the different glomerular cells, including podocytes and mesangial cells, in an intact and native environment in real-time using confocal microscopy.[4] In particular, we use 100 μm thin renal cortical slices from mice, rats and humans. After loading these with the $Ca^{2+}$ fluorescent dye fluo-4, changes in fluorescence representing changes in intracellular $Ca^{2+}$ concentrations, brought about by administration of different agonists such as angiotensin II and endothelin-1, can be captured and recorded. Further studies are underway using specific cell markers to distinguish between different glomerular cell types.

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# Diagnosing Alport syndrome using electron microscopy of the skin

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**To the Editor:** Patey-Mariaud de Serre *et al.*[1] reported that comparing α5(IV) with α2(IV) expression in skin biopsies is useful in diagnosing X-linked Alport syndrome (AS). However, α5(IV) staining variation can confuse the diagnosis and requires a different analytical approach. Morphological changes in the skin basement membrane determined using electron microscopy (EM) might provide additional information with which to diagnose AS. We found such changes using EM in a male patient with end-stage renal failure and AS. His mother had been diagnosed with chronic kidney disease and had died at the age of 58 years of malignant lymphoma. Hematoproteinuria had persisted in our patient since childhood, but a renal biopsy had not been performed. He had sensorineural hearing loss, but no ocular changes. We suspected X-linked AS and performed a skin biopsy. Standard immunofluorescence staining showed normal